



Formation of amine groups on the surface of GaN: A method for direct biofunctionalization

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ABSTRACT

Gallium nitride has attracted significant interest as a material for biosensors; however, techniques for biofunctionalizing GaN surfaces have received limited attention. Here, we present a method for producing amine groups directly on GaN surfaces through exposure to a glow discharge plasma fed with humidified air, thereby eliminating the need for complex organic functionalization chemistry. Amine formation is tracked via X-ray photoelectron spectroscopy (XPS) by labeling the plasma-formed surface groups with a fluorinated probe that binds specifically to primary amines. These amine groups can subsequently couple covalently to a wide range of biomolecules. The covalent immobilization of a NeutrAvidin layer is demonstrated using Fourier transform infrared spectroscopy (FT-IR) and fluorescent biotin-binding assays. Finally, we show that plasma exposure times sufficient for the formation of amine groups do not significantly alter the conductivity of the GaN substrate.

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1. Introduction

Gallium nitride has many characteristics that could make it an exceptional material for biosensing. It possesses desirable properties for nanowires [1–3], and is capable of both optical [2,4] and electrical [2,3] transduction. Its high piezoelectric constant also facilitates the formation of surface sensitive GaN/AlGaN two-dimensional electron gas HEMT devices [5–14]. Furthermore, GaN is robust in aqueous solutions [15] compared with other III–V materials, such as GaAs [16]. This wide array of applications, however, emphasizes the need for a reliable and reproducible method for covalently functionalizing GaN with biomolecular probes.

To date, the most prevalent functionalization method reacts a silane with the native oxide present on the GaN surface; the silane film's terminal group can then react with biomolecules using standard biofunctionalization protocols [2,8,11,17–20]. While silanization can successfully impart chemical functionality to GaN surfaces, silane films are notoriously inconsistent. Small differences in water concentration during the silanization reaction can dramatically affect the polymerization rate and

alter the thickness of the final film [21–23]. Thickness variations are particularly detrimental to GaN HEMT sensors, as they are highly sensitive to the separation between the target and the device surface. Because of these difficulties, alternative methods have been investigated, including photochemical functionalization [24], phosphonic-acid self-assembled monolayers [25,26], and gold-capping followed by thiol functionalization [4,9,10,12–14].

Recently, we published a method for the amination of silicon nitride that allows covalent coupling of proteins to the surface via the bifunctional linker glutaraldehyde (GA) [27]. Here, we show that this technique is easily extended to functionalize GaN, and may be applicable to many other nitrides. The surface amine groups are created in a radio frequency glow discharge plasma fed with humidified air. This technique allows direct coupling of biomolecules to the GaN surface, enhancing both sensor sensitivity and reproducibility by placing the molecules closer to the active electronic region of the material and eliminating poorly reproducible intermediary films such as silanes. Creation of the surface amines was examined by reaction with an amine-specific molecular label, fluorinated benzaldehyde, and characterized by X-ray photoelectron spectroscopy (XPS). Using Fourier transform infrared (FT-IR) spectroscopy and biotinylated fluorophores, we have demonstrated the subsequent attachment of NeutrAvidin (NA) using GA as a linker. Finally, the effect of the plasma treatment on the electronic response of GaN was examined using four-point resistivity measurements as a function of plasma exposure time.

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2. Experimental details

2.1. Materials

GaN wafers (6- μm thick epitaxial films on sapphire substrates) were purchased from TDI Inc. (Silver Spring, MD). Glutaraldehyde, fluorescein-labeled biotin, and 4-(trifluoromethyl)benzaldehyde (TFMB) were purchased from Sigma-Aldrich (St. Louis, MO). NeutrAvidin was purchased from Thermo Scientific (Rockford, IL).

2.2. Surface preparations

GaN wafers were cleaned for 30 s in 50% HCl, then rinsed in 18 M Ω cm de-ionized water and dried with flowing nitrogen gas. Samples were next placed in a Mercator Control Systems LF-5 radio frequency (35 kHz) glow discharge plasma system and reacted with humidity-controlled air plasma for the allotted time at a power of 135 W and a pressure of 250 mTorr. Humidified air was produced by splitting a compressed air tank feed through a bubbler and desiccant bed, and recombining the feeds at a ratio to provide 75% relative humidity. For experiments using dry air, the feed gas was run through the desiccant only (<1% relative humidity).

2.3. Fluorine labeling and XPS analysis

To determine the relative density of surface amine groups, plasma-treated samples were immersed in a 10% solution of TFMB in hexane for 2 h at room temperature. The TFMB concentration and exposure time were optimized to maximize labeling efficiency while limiting non-specific physisorption, as described previously [27]. Next, samples were sonicated in hexane for 5 min, rinsed sequentially with acetone and ethanol, and dried with flowing nitrogen gas. XPS analysis was performed using a Thermo (Waltham, MA) VG Scientific Escalab 220i-XL XPS system using an Al K α source, and data peaks were fitted using Unifit XPS analysis software. High resolution spectra were taken for the F 1s peak, and results were quantitatively compared to other samples by dividing the integrated F 1s peak intensity to the integrated Ga 3d core level peak.

2.4. Protein immobilization and fluorescent imaging

Surfaces were prepared as described in Section 2.2, plasma-treated for 60 min, and then exposed to a solution of 20% GA in de-ionized water for 2 h at room temperature. The GA solution was then aspirated, and the samples were exposed to a 2 mg/mL solution of NA in de-ionized water for 1 h at room temperature. Control samples were exposed to NA without GA exposure. Next,

all samples were rinsed with water and dried with flowing nitrogen. Finally, samples were immersed in a Petri dish containing a 1% solution of Tween-20 in water and placed on a rotator for 20 min. After this time, the samples were removed from the solution, rinsed with de-ionized water, and dried with flowing nitrogen gas. FT-IR spectra were obtained using a Thermo Nicolet 750 in single-bounce attenuated total reflectance mode and averaged over 256 scans with a resolution of 4 cm^{-1} .

For fluorescent imaging, NA-coated samples were spotted with a 5 $\mu\text{g}/\text{mL}$ solution of fluorescein-labeled biotin in 1 \times phosphate buffered saline (PBS), pH 7.4, for 30 min at room temperature. Control samples were spotted with the biotin solution without prior exposure to GA and NA. After this time, the samples were rinsed with de-ionized water, sonicated in 1 \times PBS for 1 min, rinsed again with de-ionized water, and dried with flowing nitrogen. Fluorescent images were obtained with an Olympus BX51 microscope using xenon lamp illumination and FITC-type filters (456–490 nm excitation at the sample; 510–560 nm emission collected at the camera).

2.5. Conductance measurements

Electrical contacts were patterned onto GaN samples via photolithography (200 μm \times 200 μm , with 200 μm spacing between contacts), and ohmic Ti/Al/Ni/Au contacts were deposited via e-beam metal evaporation. Following lift-off, the samples were annealed at 900 °C for 30 s in a nitrogen environment. Current-voltage (I - V) response was measured in a standard four-point configuration to remove the effects of contact resistance. I - V data were taken for a freshly cleaned sample (30 s in 50% HCl), followed by data for subsequent plasma exposure times. I - V sweeps were run over a range from -0.2 to +0.2 V. Material conductivity is calculated by dividing the slope of the I - V curve (conductance) by the contact separation distance.

3. Results and discussion

3.1. Plasma treatment and amine formation

A probable mechanism for amine formation on GaN surfaces in a humidified air plasma involves hydroxyl and free hydrogen radicals reacting with the surface, breaking Ga–N bonds to form more energetically favorable Ga–O and N–H bonds. It should be noted that this reaction scheme, while consistent with our observations, is only one possible mechanism. Detailed, real time *in situ* monitoring of the chemical composition of the surface would be required to determine a precise mechanism, and such measurements are beyond our present capability. As this proposed

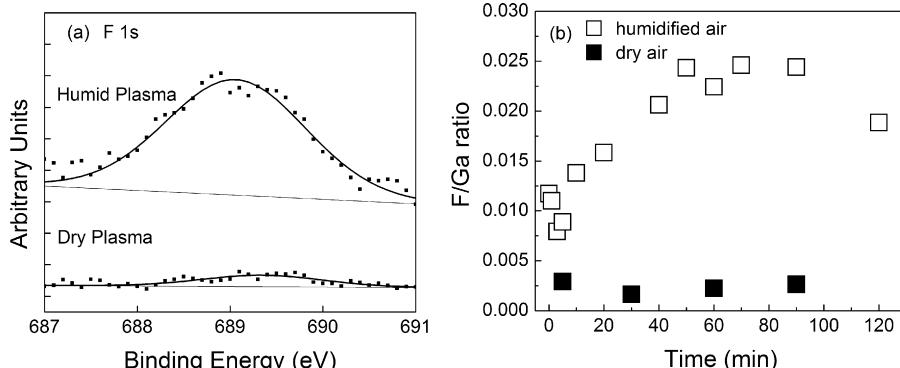


Fig. 1. (a) High resolution XPS scan of the F 1s core level peak for two GaN samples that have been exposed to air plasmas, and then exposed to a TFMB solution. The top scan is for a sample where the plasma was fed with humidified air; the bottom scan is for a sample where dry air was used. (b) Summary of fluorine peak intensity for GaN samples exposed to varying plasma reaction times and subsequently reacted with TFMB to titrate the amine groups on the surface.

mechanism necessarily leads to sub-monolayer amine coverage, an amplifying label was needed to track the formation of amine groups. Benzaldehydes have been shown previously to act as highly preferential labels for primary amines [28–30]. After plasma treatment, the samples were reacted with solutions of TFMB, and the fluorine signal from the surface-bound TFMB molecules was subsequently observable with XPS.

Fig. 1(a) shows the XPS spectra for the F 1s peak taken on a GaN sample that has undergone 60 min of exposure to humidified air plasma followed by TFMB exposure, and on a similar GaN sample that has been exposed to 60 min of dry air plasma, also followed by TFMB exposure. The figure clearly shows a strong fluorine peak for the sample exposed to the humidified air plasma, indicating TFMB binding to surface amine groups. Such a peak is not observable for the sample exposed to dry air, for which only a very small fluorine background signal is seen, most likely due to non-specific adsorption. A summary of fluorine signals (measured as integrated peak area and normalized to the Ga 3d peak) for various exposure times is given in Fig. 1(b). For the samples exposed to humidified plasma, a clear increase in TFMB binding is seen with increasing exposure time, reaching a plateau at approximately 50 min. It remains at this level until approximately 90 min of exposure, after which it begins to decline again. The decline in TFMB binding after 90 min of plasma exposure can be attributed to the fact that the continued oxidation of the GaN samples eventually removes all nitrogen from the surface, leaving only gallium oxide at the interface. This behavior is not seen for the samples exposed to dry air plasma (which lacks any hydrogen source for amine formation), with TFMB binding remaining relatively constant and near zero through 90 min of exposure. Previous studies showing the formation of surface amines on Si_3N_4 samples treated with a humidified air plasma also indicate that both substrate nitrogen and a hydrogen plasma source are necessary for substantial TFMB binding to occur [27]. Further comparison to results obtained for Si_3N_4 reveals that, for identical plasma power and levels of humidity, peak amine formation on GaN plateaus for a substantially longer period of time before beginning to decline (40 min compared to roughly only 10 min for Si_3N_4). The reason for this trend can be understood by considering the larger oxygen binding energies for Si compared to Ga. The eventual loss of surface amines to oxidation at long plasma exposure times occurs more rapidly when oxide formation is more energetically favorable; however, examination of additional nitride materials would be required to establish that the rate of oxide formation determines this trend. Another possibility for this observation may be related to the relative volatility of Ga^{1+} oxide at elevated temperatures [31]. Though a low temperature plasma was used for this procedure, there may be enough energy imparted to the surface to volatilize some of the gallium oxide, allowing exposure of the underlying nitride for longer periods of time.

3.2. Biofunctionalization

Following the amination, GaN surfaces were functionalized with the protein NeutrAvidin using glutaraldehyde as a bifunctional linker. The FT-IR spectra depicted in Fig. 2 show a sample that has been treated with humidified plasma and GA, followed by NA exposure, in comparison to a GaN sample that has simply been immersed in NA solution without GA treatment. After NA exposure, both samples were washed in 1% Tween-20 solution to remove physisorbed protein from the surface. The GA-treated sample's FT-IR spectra clearly shows amide bond peaks at 1545 and 1660 cm^{-1} that are not substantially present in the control sample, indicating the successful immobilization of a protein film [32,33]. Although the formation of specific bonds between GA and amine groups present on the surface of the protein cannot be

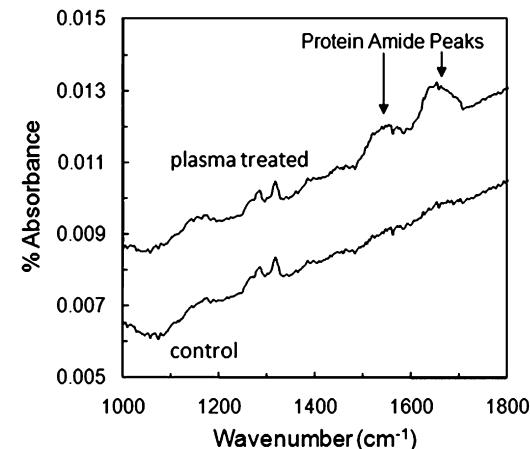


Fig. 2. FT-IR spectra for two GaN samples. The top curve is for a sample that was treated in humidified air plasma and reacted with glutaraldehyde (GA). The bottom curve is for a control sample which was plasma-treated but not exposed to GA. Both samples were then exposed to a solution of NeutrAvidin.

definitely confirmed from such a complex organic layer using FT-IR, the absence of strong amide peaks after washing the control sample shows that a relative degree of stability has been added to the plasma-treated film that is consistent with covalent bonding.

The bioactivity of the resulting protein film was demonstrated by exposure to fluorescently-labeled biotin. Fig. 3 shows fluorescent images taken for two GaN samples, one that has been plasma-treated and functionalized with GA/NA, and one control sample left untreated. Both samples were spotted with a solution containing fluorescein-tagged biotin, followed by sonication to remove physisorbed biotin from the surface. The brighter contrast

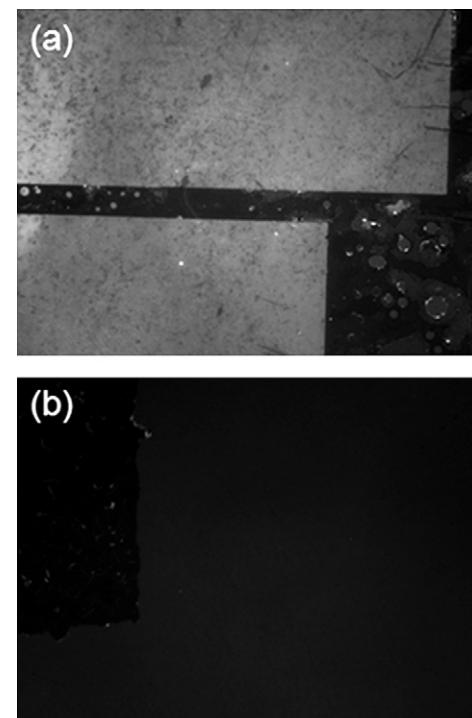


Fig. 3. Fluorescent images taken for two GaN samples, both exposed to fluorescein-tagged biotin. The top image (a) was taken for a sample that was treated with humidified air plasma and functionalized with NeutrAvidin via glutaraldehyde linkage. The bottom image (b) is of a control sample which was not biofunctionalized. The dark band seen in (a) is gold that has been patterned onto the surface to offer contrast. Image area is $350\text{ }\mu\text{m}^2$.

in Fig. 3(a) shows successful biotin-binding on the plasma-treated surface, indicating the bound NA film has maintained its function, whereas the control surface depicted in Fig. 3(b) shows no such fluorescence. Note the dark pattern seen on the functionalized sample, which is a gold feature that has been patterned onto the surface prior to plasma treatment to offer contrast. The absence of binding to the gold demonstrates the specificity of the humidified plasma functionalization for nitride surfaces.

Though the formation of surface amines through humidified plasma treatment gives inherently low amine coverage (theoretical upper limit of 33%, though most certainly less in practice), this coverage is not necessarily a detriment when considered for the immobilization of a biofilm. Previous studies have reported that approximately complete protein monolayers can be formed on surfaces by functionalizing as little as one tenth of the underlying surface with active binding sites [34]. In our previous work using humidified plasma modification of Si_3N_4 , we found via radio-labeling studies that the density of NA bound to the surface using plasma-induced amine groups did not differ significantly from protein layers attached using amine-terminated silane films, even though a higher density of surface amines exists on the silanized surface [27].

3.3. Conductivity measurements

The effect of plasma treatment on the electrical properties of GaN is of particular interest given the intended sensing mechanism. The conductivity of GaN was obtained via four-point I – V measurements and yielded linear I – V responses. A typical curve is shown in the inset of Fig. 4. The impact of plasma exposure on GaN conductivity is shown in the main plot of Fig. 4. A small, but measurable, variation of conductivity was observed. The conductivity first drops a few percent at shorter exposure times, then eventually climbs a few percent higher than the unmodified sample at longer times. The time scale for this increase in conductivity is roughly consistent with the time for peak amine formation. One explanation for this correlation is that there is an accumulation of positive charge associated with the formation of charged amine and hydroxyl groups on the surface. This would reduce the amount of positive surface depletion region charge required to maintain charge neutrality and thus reduce surface depletion depth and band bending. A smaller depletion region would lead to an increase in conductivity which is consistent with the data of Fig. 4. It should be noted, however, that such a small variation in conductivity ($\sim 2\%$) is not expected to greatly affect the operation of a device.

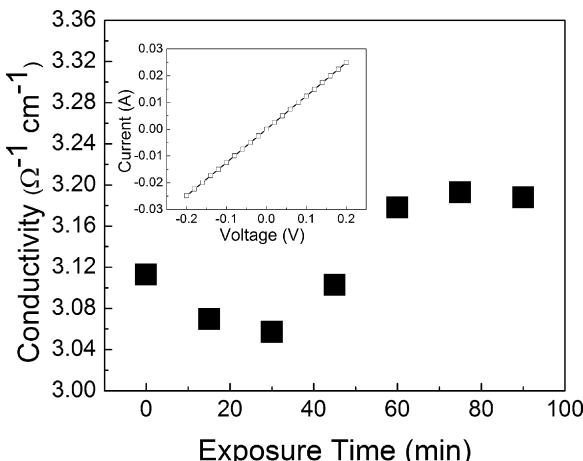


Fig. 4. Impact of plasma treatment on conductivity. Inset shows a typical I – V curve. The bulk conductivity of the samples vary by only $\sim 2\%$ from an untreated control.

4. Conclusions

We have presented a simple method for forming amine groups directly on the surface of GaN which allows for direct biofunctionalization without intermediary films such as silanes. Amine groups were formed on the surface of GaN through exposure to a humidified air plasma. The presence of these groups was verified with XPS and relative density tracked as a function of plasma exposure time. The amine groups were used, in conjunction with glutaraldehyde, to covalently attach a layer of NeutrAvidin. It was also shown that the bound NA film retained biological activity through its interaction with fluorescently-labeled biotin. The treatment of the GaN surface in humidified plasma did not appreciably alter the bulk conductivity of the substrate, suggesting that this treatment should be applicable for GaN-based biodetection schemes, particularly those where the surface proximity of the probe molecules is significant.

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References

- [1] B.S. Simpkins, K.M. McCoy, L.J. Whitman, P.E. Pehrsson, *Nanotechnology* 18 (2007) 355301.
- [2] C.P. Chen, A. Ganguly, C.-H. Wang, C.-W. Hsu, S. Chattopadhyay, Y.-K. Hsu, Y.-C. Chang, K.-H. Chen, L.-C. Chen, *Anal. Chem.* 81 (2009) 36.
- [3] Y. Huang, X. Duan, Y. Cui, C.M. Leiber, *Nano Lett.* 2 (2002) 101.
- [4] H.Y. Shih, T.T. Chen, C.H. Wang, K.Y. Chen, Y.F. Chen, *Appl. Phys. Lett.* 92 (2008) 261910.
- [5] B. Lubbers, G. Kittler, P. Ort, S. Linkohr, D. Wegener, B. Baur, M. Gebinoga, F. Weise, M. Eickhoff, S. Maroldt, A. Schober, O. Ambacher, *Phys. Status Solidi C* 5 (2008) 2361.
- [6] G. Steinhoff, O. Purrucker, M. Tanaka, M. Stutzmann, M. Eickhoff, *Adv. Funct. Mater.* 13 (2003) 841.
- [7] G. Steinhoff, B. Baur, G. Wrobel, S. Ingebrandt, A. Offenhausser, A. Dadgar, A. Krost, M. Stutzmann, M. Eickhoff, *Appl. Phys. Lett.* 86 (2005) 033901.
- [8] B. Baur, H.-G. von Ribbeck, Y. Gawlina, V. Bandalo, G. Steinhoff, M. Stutzmann, M. Eickhoff, *Appl. Phys. Lett.* 89 (2006) 183901.
- [9] K.H. Chen, B.S. Kang, H.T. Wang, T.P. Lele, F. Ren, Y.L. Wang, C.Y. Chang, S.J. Pearton, D.M. Dennis, J.W. Johnson, R. Rajagopal, J.C. Roberts, E.L. Piner, K.J. Linthicum, *Appl. Phys. Lett.* 92 (2008) 192103.
- [10] H.T. Wang, B.S. Kang, F. Ren, S.J. Pearton, J.W. Johnson, P. Rajagopal, J.C. Roberts, E.L. Piner, K.J. Linthicum, *Appl. Phys. Lett.* 91 (2007) 222101.
- [11] B.S. Kang, F. Ren, L. Wang, C. Lofton, W.W. Tan, S.J. Pearton, A. Dabiran, A. Osinsky, P.P. Chow, *Appl. Phys. Lett.* 87 (2005) 023508.
- [12] B.S. Kang, S.J. Pearton, J.J. Chen, F. Ren, J.W. Johnson, R.J. Therrien, P. Rajagopal, J.C. Roberts, E.L. Piner, K.J. Linthicum, *Appl. Phys. Lett.* 89 (2006) 122102.
- [13] B.S. Kang, H.T. Wang, T.P. Lele, Y. Tseng, F. Ren, S.J. Pearton, J.W. Johnson, P. Rajagopal, J.C. Roberts, E.L. Piner, K.J. Linthicum, *Appl. Phys. Lett.* 91 (2007) 112106.
- [14] B.S. Kang, H.T. Wang, F. Ren, S.J. Pearton, *J. Appl. Phys.* 104 (2008) 031101.
- [15] D. Zhuang, J.H. Edgar, *Mater. Sci. Eng. R* 48 (2005) 1.
- [16] D.E. Aspnes, A.A. Studna, *Appl. Phys. Lett.* 46 (1985) 1071.
- [17] A. Arranz, C. Palacio, D. Garcia-Fresnadillo, G. Orellana, A. Navarro, E. Munoz, *Langmuir* 24 (2008) 8667.
- [18] B. Baur, G. Steinhoff, J. Hernando, O. Purrucker, M. Tanaka, B. Nickel, M. Stutzmann, M. Eickhoff, *Appl. Phys. Lett.* 87 (2005) 263901.
- [19] R.M. Petoral, G.R. Yazdi, A.L. Spetz, R. Yakimova, K. Uvdal, *Appl. Phys. Lett.* 90 (2007) 223904.
- [20] R. Yakimova, G. Steinhoff, R.M. Petoral, C. Vahlberg, V. Khranovskyy, G.R. Yazdi, K. Uvdal, A.L. Spetz, *Biosens. Bioelectron.* 22 (2007) 2780.
- [21] D.F.S. Petri, G. Wenz, P. Schunk, T. Schimmel, *Langmuir* 15 (1999) 4520.
- [22] A. Simon, T. Cohen-Bouhacina, M.C. Porte, J.P. Aime, C. Baquey, *J. Colloid Interface Sci.* 251 (2002) 278.
- [23] A. Wang, H. Tang, T. Cao, S.O. Salley, K.Y.S. Ng, *J. Colloid Interface Sci.* 291 (2005) 438.
- [24] H. Kim, P.E. Colavita, K.M. Metz, B.M. Nichols, B. Sun, J. Uhrlrich, X. Wang, T.F. Kuech, R.J. Hamers, *Langmuir* 22 (2006) 8121.

- [25] T. Ito, S.M. Forman, C. Cao, F. Li, C.R. Eddy, A. Mastro, R.T. Holm, R.L. Henry, K.L. Hohn, J.H. Edgar, *Langmuir* 24 (2008) 6630.
- [26] H. Kim, P.E. Colavita, P. Paoprasert, P. Gopalan, T.F. Kuech, R.J. Hamers, *Surf. Sci.* 602 (2008) 2382.
- [27] R. Stine, C.L. Cole, K.M. Ainslie, S.P. Mulvaney, L.J. Whitman, *Langmuir* 23 (2007) 4400.
- [28] I. Keen, P. Broota, L. Rintoul, P. Fredericks, M. Trau, L. Grondahl, *Biomacromolecules* 7 (2006) 427.
- [29] M. Nitschke, G. Schmack, A. Janke, F. Simon, D. Pleul, C. Werner, *J. Biomed. Mater. Res.* 59 (2002) 632.
- [30] B. Olander, A. Wirsén, A.C. Albertsson, *Biomacromolecules* 4 (2003) 145.
- [31] Z.R. Wasilewski, J.M. Baribeau, M. Beaulieu, X. Wu, G.I. Sproule, *J. Vac. Sci. Technol. B* 22 (2004) 1534.
- [32] R. Stine, M.V. Pishko, C.L. Schengrund, *Langmuir* 20 (2004) 6501.
- [33] R. Stine, M.V. Pishko, C.L. Schengrund, *Anal. Chem.* 77 (2005) 2882.
- [34] B.L. Frey, C.E. Jordan, S. Kornguth, R.M. Corn, *Anal. Chem.* 67 (1995) 4452.